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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: John Kisiday et al.

Art Unit: 1614

Serial No.: 09/778,200

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Title: PEPTIDE SCAFFOLD ENCAPSULATION OF TISSUE CELLS AND
USES THEREOF

Assistant Commissioner for Patents
Washington, D.C. 20231

Version with Markings to Show Changes Made

A marked-up version of Table 1 on page 19, line 1, through page 20, line 9, of the specification is presented below.

Table 1. Representative Self-Assembling Peptides

Name	Sequence (n-->c)	Modulus	Structure	SEQ ID NO
RADA16-I	n-RADARADARADARADA-c	I	β	<u>1</u>
RGDA16-I	n-RADARGDARADARGDA-c	I	r.c.	<u>2</u>
RADA8-I	n-RADARADA-c	I	r.c.	<u>3</u>
RAD16-II	n-RARADADARARADADA-c	II	β	<u>4</u>
RAD8-II	n-RARADADA-c	II	r.c.	<u>5</u>
EAKA16-I	n-AEAKAEAKAEAKAEAK-c	I	β	<u>6</u>
EAKA8-I	n-AEAKAEAK-c	I	r.c.	<u>7</u>
RAEA16-I	n-RAEARAEARAEARAEA-c	I	β	<u>8</u>
RAEA8-I	n-RAEARAEA-c	I	r.c.	<u>9</u>
KADA16-I	n-KADAKADAKADAKADA-c	I	β	<u>10</u>
KADA8-I	n-KADAKADA-c	I	r.c.	<u>11</u>
EAH16-II	n-AEAEAHAAHAEAEAHAAH-c	II	β	<u>12</u>
EAH8-II	n-AEAEAHAAH-c	II	r.c.	<u>13</u>
EFK16-II	n-FEFEFKFKFEFEFKFK-c	II	β	<u>14</u>
EFK8-II	n-FEFKFEFK-c	I	β	<u>15</u>
ELK16-II	n-LELELKLKLELELKLK-c	II	β	<u>16</u>
ELK8-II	n-LELELKLK-c	II	r.c.	<u>17</u>
EAK16-II	n-AEAEAKAKAEAEAKAK-c	II	β	<u>18</u>
EAK12	n-AEAEAEAEAKAK-c	IV/II	α/β	<u>19</u>
EAK8-II	n-AEAEAKAK-c	II	r.c.	<u>20</u>
KAE16-IV	n-KAKAKAKAEAEAEAEA-c	IV	β	<u>21</u>
EAK16-IV	n-AEAEAEAEAKAKAKAK-c	IV	β	<u>22</u>
RAD16-IV	n-RARARARADADADADA-c	IV	β	<u>23</u>
DAR16-IV	n-ADADADADARARARAR-c	IV	α/β	<u>24</u>
DAR16-IV*	n-DADADADARARARARA-c	IV	α/β	<u>25</u>
DAR32-IV	n-(ADADADADARARARAR)-c	IV	α/β	<u>26</u>
EHK16	n-HEHEHKHKHEHEHKHK-c	N/A	r.c.	<u>27</u>
EHK8-I	n-HEHEHKHK-c	N/A	r.c.	<u>28</u>
VE20*	n-VEVEVEVEVEVEVEVEVE-c	N/A	β	<u>29</u>
RF20*	n-RFRFRFRFRFRFRFRFRF-c	N/A	β	<u>30</u>

“ β ” denotes beta-sheet; “ α ” denote alpha-helix; “r.c.” denotes random coil; “N/A” denotes not applicable. *Both VE20 and RF20 form a beta-sheet when they are incubated in a solution containing NaCl; however, they do not self-assemble to form macroscopic scaffolds.

A marked-up version of Table 2 on page 29, lines 17 to 25, of the specification is presented below.

Table 2. Representative Peptides for Cross-Linking Study

<u>Name</u>	<u>Sequence (N-->C)</u>	<u>SEQ ID. NO.</u>
RGDY16	RGDYRYDYRYDYRGDY	<u>31</u>
RGDF16	RGDFRFD FRDFRGDF	<u>32</u>
RGDW16	RGDWRWDWRWDWRGDW	<u>33</u>
RADY16	RADYRYEYRYEYRADY	<u>34</u>
RADF16	RADFRFD FRDFRADF	<u>35</u>
RADW16	RADWRWDWRWDWRADW	<u>36</u>

A marked-up version of Table 3 on page 22, lines 14 to 21, of the specification is presented below.

Table 3. Representative Peptides for Enzymatic Cleavage Study

<u>Name</u>	<u>Sequence (N-->C)</u>	<u>SEQ ID NO</u>
REEE	RGDYRYDYTFREEE-GLGSRYDYRGDY	<u>37</u>
KEEE	RGDYRYDYTFKEEE-GLGSRYDYRGDY	<u>38</u>
SELE	RGDYRYDYTASELE-GRGTRYDYRGDY	<u>39</u>
TAQE	RGDYRYDYAPTAQE-AGEGPRYDYRGDY	<u>40</u>
ISQE	RGDYRYDYPTISQE-LGQRPRYDYRGDY	<u>41</u>
VSQE	RGDYRYDYPTVSQE-LGQRPRYDYRGDY	<u>42</u>

A marked-up version of the paragraph on page 23, line 22, through page 24, line 14, of the specification is presented below.

“A peptide with the amino acid sequence n-KLDLKLDLKLDL-c (SEQ ID NO. 43) (KLD12) was synthesized using a peptide synthesizer (Applied Biosystems) and lyophilized to a powder. A 0.5% peptide casting solution was obtained by dissolving KLD12 in a solution of 295 mM sucrose and 1 mM HEPES. Freshly isolated chondrocytes from bovine calf femoropatellar groove cartilage were re-suspended in the casting solution at a concentration of 15×10^6 cells/ml. The suspension was injected into a

casting frame consisting of a 40 x 40 x 1.5 mm window supported on both faces by filter paper and a porous mesh. The casting frame was placed in a 1 X phosphate-buffered saline (PBS, which contains 150 mM NaCl and 10 mM sodium phosphate at pH 7.4) bath for 15 minutes to induce the self-assembly of the peptides into a scaffold. Preferably, the cells are incubated in the sucrose solution for less than 5 minutes, or more preferably for less than 1 minute, before PBS is added. If desired, formation of a peptide scaffold may be confirmed using phase-contrast microscopy. As a control, cells were also suspended into warm agarose (2% solution, w/w), injected into the casting frame, and placed into a cold 1 X PBS bath for 5 minutes. Both the peptide and control agarose gels were maintained in DMEM media (Gifco) plus 10 % FBS, which was changed every other day."